Claims

1. An isolated nucleic acid molecule, comprising a nucleic acid sequence comprising at least 50 nucleotides
   a) of the nucleic acid sequence of one of Figs. 21A, B,
   b) of an allele of the nucleic acid sequence of one of Figs. 21A, B, or
   c) of a nucleic acid sequence which under stringent conditions hybridizes with the nucleic acid sequence of one of Figs. 21A, B.

2. The nucleic acid molecule according to claim 1, wherein said nucleic acid sequence comprises at least 50 nucleotides
   a) of the nucleic acid sequence of one of Figs. 22A - Q,
   b) of a nucleic acid sequence, which corresponds to the nucleic acid sequence of one of Figs. 22A - Q within the degeneration of the genetic code, or
   c) of a nucleic acid sequence which under stringent conditions hybridizes with the nucleic acid sequence of one of Figs. 22A - Q.

3. The nucleic acid molecule according to claim 2, wherein said nucleic acid sequence comprises
   a) the nucleic acid sequence of one of Figs. 22A - Q,
   b) a nucleic acid sequence, which corresponds to the nucleic acid sequence of one of Figs. 22A - Q within the degeneration of the genetic code, or

c) of a nucleic acid sequence which under stringent conditions hybridizes with the nucleic acid sequence of one of Figs. 22A - Q.

4. The nucleic acid molecule according to any one of the claims 1 to 3, wherein at least one coding region is functionally deleted.

5. The nucleic acid molecule according to any one of the claims 1 to 4, having inserted therein at least one insertion cassette for transposon or phage mediated insertion.

6. The nucleic acid molecule according to any one of the claims 1 to 5, wherein at least one heterologous nucleic acid molecule coding for a polypeptide or peptide is inserted or deletion-inserted.

7. The nucleic acid molecule according to claim 7, wherein the sequences flanking said heterologous nucleic acid molecule each have a length of at least 50 nucleotides, preferred 200 - 250 nucleotides.

8. The nucleic acid molecule according to claim 6 or 7, wherein said heterologous nucleic acid molecule comprises a nucleic acid sequence coding for a bacterial or viral antigen or homologue thereof.

9. The nucleic acid molecule according to claim 6 or 7, wherein said heterologous nucleic acid molecule comprises a nucleic acid sequence coding for a tumor antigen.

10. The nucleic acid molecule according to any one of the claims 7 to 9, wherein said heterologous nucleic acid molecule comprises at least one gene expression cassette.

11. The nucleic acid molecule according to any one of the claims 7 to 10, wherein said heterologous nucleic acid molecule comprises at least one transactivator cassette, selective marker cassette, invertase cassette or combination thereof.

12. The nucleic acid molecule according to any one of the claims 7 to 11, wherein said heterologous nucleic acid molecule comprises at least one nucleic acid sequence coding for a polypeptide or peptide targeting and/or immunostimulatory domain.

13. A recombinant vector comprising the nucleic acid molecule according to any one of the claims 1 to 12.

14. A cell comprising the nucleic acid molecule according to any one of the claims 5 to 12 or the recombinant vector according to claim 13.

15. The cell according to claim 14, wherein the cell is a gram-negative cell.

16. The cell according to claim 14, wherein the cell is a Salmonella cell.

17. A peptide or polypeptide comprising a peptide sequence comprising at least 20 amino acids

a) of the sequence of Figs. 23A - Q, or

b) of a sequence which is 60 % homologous to the sequence of Figs. 23A - Q.

18. A polypeptide comprising the sequence

a) of Figs. 23A - Q, or

b) which is 60 % homologous to the sequence of Figs. 23A-Q.

19. An antibody directed against the polypeptide according to any one of the claims 17 and 18.

20. A fusion protein comprising the polypeptide according to any one of the claims 17 and 18 having inserted or deletion-inserted or being fused C- or NH_2-terminally with at least one heterologous polypeptide.

21. The fusion protein according to claim 20, wherein the heterologous polypeptide is selected from bacterial, viral or tumor antigens.

22. An attenuated gram-negative cell comprising the SPI2 gene locus, wherein at least one gene of the SPI2 locus is inactivated, wherein said inactivation results in an attenuation/reduction of virulence compared to the wild type of said cell.

23. The attenuated gram-negative cell according to claim 22, wherein at least one inactivated gene is selected from effector (sse) genes, secretion apparatus (ssa) genes, chaperon (ssc) genes and regulation (ssr) genes.

24. The attenuated gram-negative cell according to claims 22 and 23, wherein said cell is an Enterobactericae cell, in particular, a Salmonella cell, a Shigella cell or a Vibrio cell.

25. The attenuated gram-negative cell according to claim 24, wherein said cell is a Salmonella cell.

26. The attenuated gram-negative cell according to claim 24 or 25, wherein said cell has a broad host range.

27. The attenuated gram-negative cell according to claim 26, wherein said cell is a Salmonella serotype Typhimurium Definitive Type 104 (DT104) cell.

28. The cell according to any one of the claims 22 to 27, wherein at least one inactivated gene is selected from sse, ssc and ssr.

29. The cell according to any one of the claims 22 to 28, wherein at least one inactivated gene comprises at least one sse gene.

30. The cell according to claim 29, wherein at least one sse gene is selected from sseC, sseD and sseE.

31. The cell according to any one of the claims 22 to 30, wherein at least one inactivated gene comprises at least one ssr gene.

32. The cell according to claim 31, wherein said at least one ssr gene is ssrB.

33. The cell according to any one of the claims 22 to 32, wherein at least one inactivated gene comprises at least one ssc gene.

34. The cell according to claim 33, wherein said at least one ssc gene is sscB.

35. The cell according to any one of the claims 22 to 34, wherein at least one gene is inactivated by a mutation comprising a deletion.

36. The cell according to claim 35, wherein said deletion comprises at least 6 nucleotides.

37. The cell according to any one of the claims 35 and 36, wherein the mutation comprises a deletion of the complete coding sequence for said gene.

38. The cell according to any one of the claims 22 to 37, wherein at least one gene is inactivated by a mutation comprising the insertion of a heterologous nucleic acid molecule.

39. The cell according to any one of the claims 35 to 38, wherein said mutation is a non-polar mutation.

40. The cell according to any one of the claims 22 to 39, wherein at least one additional gene located outside of the SPI2 locus is inactivated,

wherein the inactivation results in a further attenuation/reduction of virulence compared to the wild type.

41. The cell according to claim 40, wherein said additional gene comprises an aro gene.

42. The cell according to claim 41, wherein said aro gene is aro A.

43. The cell according to claim 40, wherein said additional gene is superoxide dismutase.

44. The cell according to any one of the claims 22 to 43, comprising at least one selective marker cassette.

45. The cell according to claim 44, wherein said selective marker cassette is capable of conferring an antibiotic resistance to the cell.

46. The cell according to any one of the claims 22 to 45 comprising at least one gene expression cassette.

47. The cell according to any one of the claims 22 to 46 comprising at least one transactivator cassette.

48. The cell according to any one of the claims 22 to 47 comprising at least one invertase cassette.

49. The cell according to any one of the claims 22 to 48 comprising at least one insertion cassette.

50. A carrier for the presentation of an antigen to a host, which carrier is an attenuated gram-negative cell according to any one of the claims 22 to 49, wherein said cell comprises at least one heterologous nucleic acid molecule comprising a nucleic acid sequence coding for said antigen, wherein said cell is capable of expressing said nucleic acid molecule or capable of causing the expression of said nucleic acid molecule in a target cell.

51. The carrier according to claim 50, wherein said nucleic acid molecule comprises a nucleic acid sequence coding for a bacterial or viral antigen or a tumor antigen.

52. The carrier according to claim 51, wherein said nucleic acid sequence codes for an antigen from Helicobacter pylori, Chlamydia pneumoniae,

Borrelia burgdorferi, Nanobacteria, Hepatitis virus, human papilloma virus or Herpes virus.

53. The carrier according to any of the claims 50 to 52, wherein said nucleic acid molecule is inserted into the SPI2 locus.

54. The carrier according to claim 53, wherein said nucleic acid molecule is inserted into an sse gene.

55. The carrier according to claim 54, wherein said sse gene is selected from sseC, sseD and sseE.

56. The carrier according to any one of the claims 50 to 55, wherein said insertion is a non-polar insertion.

57. The carrier according to any one of the claims 50 to 56, wherein the expression of said heterologous nucleic acid molecule is tissue specific.

58. The carrier according to any one of the claims 50 to 57, wherein the expression of said heterologous nucleic acid is inducible.

59. The carrier according to any one of the claims 50 to 58, wherein the expression of said heterologous nucleic acid is activated in a target cell.

60. The carrier according to claim 59, wherein said target cell is a macrophage.

61. The carrier according to any of the claims 50 to 60, wherein said nucleic acid molecule comprises a nucleic acid sequence coding for a least one polypeptide or peptide targeting and/or immunostimulatory domain.

62. The carrier according to any one of the claims 50 to 60, wherein said nucleic acid molecule codes for a fusion protein.

63. The cell according to any one of the claims 50 to 62, wherein the expression product of said nucleic acid molecule remains in the cytosole of said carrier.

64. The carrier according to any one of the claims 50 to 62, wherein the expression product of said nucleic acid molecule is directed to the periplasmatic space of said carrier.

65. The carrier according to any one of the claims 50 to 62, wherein the expression product of said nucleic acid molecule is directed to the outer membrane of said carrier.

66. The carrier according to any one of the claims 50 to 62, wherein the expression product of said nucleic acid molecule is secreted.

67. The carrier according to claim 66, wherein the expression product of said nucleic acid molecule is secreted by the type III secretion system.

68. The carrier according to claim 67, wherein the expression product of said nucleic acid molecule is secreted by the SPI2 type III secretion system.

69. An attenuated gram-negative cell comprising the SPI2 gene locus, characterized by a lack of at least one SPI2 polypeptide, wherein said lack results in an attenuation/reduction of virulence compared to the wild type of said cell.

70. The attenuated gram-negative cell according to claim 69, wherein said missing polypeptide is selected from effector (sse) polypeptides, secretion apparatus (ssa) polypeptides, chaperon (ssc) polypeptides and regulatory (ssr) polypeptides.

71. A carrier for the presentation of an antigen to a host, which carrier is an attenuated gram-negative cell according to claims 69 or 70, further characterized by the presence of at least one heterologous peptide or polypeptide having immunogenic properties.

72. A pharmaceutical composition, comprising as an active agent an immunologically protective living vaccine, which is an attenuated cell according to any one of the claims 22 to 49, 69 and 70 or a carrier according to any one of the claims 50 to 68 and 71.

73. The composition according to claim 72 together with pharmaceutically acceptable diluents, carriers and/or adjuvants.

74. The composition according to any one of the claims 72 and 73, which is suitable for administration to a mucosal surface or via the parenteral route.

75. A method for the preparation of a living vaccine, comprising providing a living gram-negative cell comprising the SPI2 locus and inactivating at least one gene of the SPI2 locus to obtain an attenuated gram-negative cell according to any one of the claims 22 to 49, 69 and 70.

76. The method of claim 75, further comprising inserting at least one heterologous nucleic acid molecule coding for an antigen to obtain a carrier according to any one of the claims 50 to 68 and 71.

77. A method for the preparation of a living vaccine composition comprising formulating an attenuated cell according to any one of the claims 22 to 49, 69 and 70 or a carrier according to any one of the claims 50 to 68 and 71 in a pharmaceutically effective amount together with pharmaceutically acceptable diluents, carriers and/or adjuvants.

78. A method for the detection of an attenuated cell according to any one of the claims 22 to 49, 69 and 70 or a carrier according to any one of the claims 50 to 68 and 71, comprising providing a sample containing said cell and detecting a specific property not present in wild type.

79. A method for establishing a library of attenuated gram-negative cells comprising obtaining at least two attenuated gram-negative cells according to any one of the claims 22 to 49, 69 and 70, determining the pathogenicities of said cells, and determining the relation of the pathogenicities of said cells.

80. The method according to claim 79 further comprising determining the immunogenicities of said cells and determining the relation of the immunogenicities of said cells.

81. The method of claim 80, wherein the determination of the immunogenicity is a determination of the humoral, cellular and/or mucosal immunogenicity.

82. A method for establishing a library of attenuated carriers for the presentation of an antigen to a host, comprising obtaining at least two carriers according to any one the claims 50 to 68 and 71, determining (a) the pathogenicities of said cells and the relation of the pathogenicities of said cells, and (b) determining the effect of said antigen presentation in said host and determining the relation of said effects caused by said cells.

83. The method of claim 82, wherein the effect of antigen presentation is determined at humoral, cellular and/or mucosal level.

84. The method according to claim 82 or 83 further comprising determining the immunogenicities of said cells and determining the relation of the immunogenicities of said cells, wherein said determination optionally is a determination of the humoral, cellular and/or mucosal immunogenicity.

85. The use of an attenuated cell according to any one of the claims 22 to 49, 69 and 70 or a carrier according to any one of the claims 50 to 68 and 71 for the preparation of a drug for the preventive or therapeutic

treatment of an acute or chronic disease caused essentially by a bacterium or virus.

86. The use according to claim 85, wherein said disease is caused essentially by a Salmonella cell.

87. The use of a carrier according to any one of the claims 50 to 68 and 71 for the preparation of a drug for the preventive or therapeutic treatment of a tumour.

88. The use of a nucleic acid molecule according to any one of the claims 1 to 12 or a vector according to claim 13 for the preparation of an attenuated cell, a living vaccine or a carrier for the presentation of an antigen to a host.

89. Use of the Salmonella SPI2 locus for the preparation of an attenuated cell, a living vaccine or a carrier for the presentation of an antigen to a host.

90. Use of a virulence gene locus of a gram-negative cell for the preparation of a carrier for the presentation of an antigen to a host.

91. An isolated nucleic acid molecule comprising a nucleic acid of at least 100 nucleotides

a) of the nucleic acid sequence of one of Figs.24A, B

b) of a nucleic acid sequence which under stringent conditions hybridizes with the nucleic acid sequence of one of Figs.24A, B.

92. The nucleic acid molecule according to claim 91, wherein said nucleic acid molecule is capable of inducing the expression of a nucleic acid sequence coding for a peptide or polypeptide operatively linked to said nucleic acid molecule.

93. Expression system for the in-vivo inducible expression of a heterologous nucleic acid in a target cell, comprising a carrier cell for said heterologous nucleic acid, wherein said carrier cell comprises (a) a polypeptide having the amino acid sequence shown in Figure 23P (ssrA) or a functional homologue thereof, (b) a polypeptide having the amino acid sequence shown in Figure 23Q (ssrB) or a functional homologue thereof, and (c) the nucleic acid molecule according to claim 92.

94. Expression system according to claim 91, wherein said target cell is a macrophage.

95. Expression system according to claims 93 and 94, wherein said carrier cell is a Salmonella cell.

96. The expression system according to any of the claims 93 to 95, wherein said target cell comprises a gene expression cassette.

97. The expression system according to any of the claims 93 to 96, wherein said target cell comprises an insertion cassette.

98. The expression system according to any of the claims 93 to 97, wherein said target cell comprises a heterologous nucleic acid molecule coding for a peptide or polypeptide.

99. Use of the nucleic acid molecule according to claim 92 for the in vivo inducible expression of a heterologous nucleic acid molecule.

100. Use of the nucleic acid molecule according to claim 92 for the detection of in vivo inducible promoters.